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SOME SOIL CHANGES PRODUCED BY MICRO-ORGANISMS

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By WALTER G. SACKETT



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SOME SOIL CHANGES PRODUCED BY MICRO-ORGANISMS

By Walter G. Sackett

The prosperous farmer has learned through the school of experience that if he follows certain practices he can keep his land in a productive condition. This practical knowledge is of inestimable value, but it does not go far enough. In order to be able to proceed intelligently and to meet problems out of the ordinary routine, when they arise, he should know more about the fundamental principles which underlie his profession. While there are many factors concerned in the conservation of plant food in the soil, the part which micro-organisms play is among the most important, and in the pages which follow, it is the purpose of the writer to present as clearly as possible a story of the activities of soil bacteria, yeasts, and molds.

Before passing to a consideration of the work which bacteria perform in the soil, it may be well to learn something of the nature of these micro-organisms themselves.

THE NATURE OF BACTERIA.

In the year 1675, Anton van Leeuwenhoek, a skilled lens maker of Delft, Holland, observed for the first time what we know today as bacteria. He devoted much of his time to examining through the microscope such material as was especially suited to testing his lenses, and on the occasion mentioned, he chanced to be looking at some tartar scraped from the teeth and mixed with a little water. To his great surprise and wonder, Leeuwenhoek saw what he believed to be, "many tiny animals which moved about in a most amusing fashion." He described the different shapes and supplemented his observations with drawings which resemble closely the present day forms. He was mistaken, however, in interpreting what he found as animal life, for the careful study of recent years has shown that bacteria or germs belong to the plant kingdom. To be sure, they are different from the plants with which most of us are familiar, but their food habits, the chemical composition of some of them, their simple shapes and their relation to such recognized plant forms as the molds and yeasts place them unquestionably with the lower forms of vegetable life.

Much of our knowledge of these organisms remained locked up

in the mysteries of speculation and conjecture for nearly two hundred years, until finally in 1882, Dr. Robert Koch, a German bacteriologist, discovered a means of growing bacteria outside of their natural environment. While great progress has been made in the science of bacteriology since that time, and numerous new and highly specialized methods of cultivating germs have been devised, yet it is interesting to note, in passing, that many of these depend upon the fundamental principles demonstrated by Koch, and without his splendid achievements, they might never have been possible.

The public has been extremely unfortunate in the way in which it has acquired a great deal of its knowledge of bacteria. Much of this information has come to us through the medium of patent medicine advertisements, quack medical advice, and testimonials for worthless water filters. What one of my readers, while consulting the family almanac to find the time of the next new moon, has not paused for a moment to look at some hideous picture, purporting to be the exact likeness of the germs of some terrible disease, which can be cured absolutely by taking two bottles of So and So's Bitters? Our conception of bacteria as gained from this sort of rubbish is as far from the truth as the east is from the west. Everything from an innocent cray fish to a writhing sea serpent with yawning jaws, gnashing teeth and a lashing tail is herded into these illustrations to send a thrill of horror over the victim who reads his fate.

Bacteria are very simple in shape, and can be divided on this basis into three general classes. An easy way to remember the three different groups is to think of them as resembling in outline lead pencils, marbles and cork screws, although, of course, so small as to be seen only by the aid of a powerful magnifying glass. (See Plate I.) The bacteriologist knows the lead pencil or rod type as a *bacillus*, the marble or spherical one as a *coccus*, and the cork screw, curved or comma form as a *spirillum*. The bacillus group has the largest membership of the three, and includes such germs as produce typhoid fever, diphtheria, tuberculosis, anthrax, lock jaw, pear blight, alfalfa blight, potato wilt, black rot of cabbage, bitter milk, sauer kraut, vinegar, etc.; next in numbers come the cocci (pronounced coxi), represented by the germs of pneumonia, sore throat, erysipelas, boils and pus; the spirilla constitute the smallest division, in which we find the germs of Asiatic cholera, syphilis and relapsing fever. These are only a few of the hundreds that are known, but they will serve to illustrate the classification.

Next, let us see something about the size of these tiny plants.

It will be impossible in an article of this kind to tell how they are measured, although the process is comparatively simple. The reader will have to take the writer's word for it that it can be done and with a remarkable degree of accuracy. Some of the cocci or spherical forms are so small that it has been estimated that one billion could easily be contained in a drop of water having a volume of about one fortieth of a teaspoonful. With many of the rods or bacilli, we should need fifteen to twenty thousand individuals placed end to end to make one inch. The largest bacteria, as a rule, are found among the spirilla. These vary in length from one twelve thousandth to one six hundred twenty-fifth of an inch and from one fifty thousandth to one seven thousandth of an inch in diameter, while the bacilli average around one fifteen thousandth of an inch long by one thirty thousandth of an inch in diameter.

Although extremely small, bacteria make up for this deficiency by their tremendous numbers, through which agency they are capable of accomplishing wonderful results, both as friends and foes, in a remarkably short time. Multiplication takes place, by a process called fission, once about every twenty minutes to a half hour and proceeds by a geometrical progression. Thus, if we have a single living germ at the present moment, it will increase in size, and at the end of twenty minutes, will divide in the middle by the formation of a cross partition (fission), and each half will become a complete organism in itself. We thus have two germs; these two again divide by the same process so that after twenty minutes more, there are four and then eight, sixteen, thirty-two, sixty-four, etc. If there were no forces to check this growth, it would be difficult, indeed, to predict just what would become of the rest of us here upon the earth; but, fortunately, multiplication is controlled by the excretions of the germs themselves. It has been estimated that if division took place once every twenty minutes, and that it proceeded at this rate uninterrupted for twenty-four hours, the descendants of one germ would number 1,806,655,286,826,042,269,696, or, in round numbers, 1,800,000 trillions. Considering that each organism was one fifteen thousandth of an inch long, the above number, if placed end to end, would make a string nearly two trillion miles long; it would reach to the sun and back again 10,000 times, would go around the earth's equator 70,000,000 times, and it would take a ray of light 100,000 years to pass from one end of it to the other.

The parent cell and all of its descendants constitute a bacterial colony, and when the microorganism is grown on a suitable food

stuff so that all of the offspring is retained in one place, the colony first appears to the naked eye as a tiny speck which grows to the size of a pin head or larger depending upon the species and the environmental conditions. (See Plates II and III.)

Any growth of bacteria, yeasts or molds is spoken of as a *culture*; if this is made up of several different kinds of organisms, it is referred to as a *mixed culture* (Plate III, 2.), but if it is composed of only one species, then it is termed a *pure culture* (Plate III, 1). Thus, we have pure culture starters for butter, cheese and vinegar.

Although we do not ordinarily think of plants as being able to move about, some bacteria, notably the rods and spirals, possess this power in a very marked degree. They are provided with very fine, hair-like appendages, known as flagella, which extend outward from the body of the cell like the legs of a "grand-daddy-long-legs." Sometimes they come out from all sides of the germ and again only from the ends. These are kept moving rapidly all of the time with a lashing motion, much as a fish moves its fins. and by this means the bacteria are able to swim from place to place through the body fluids, decaying matter, milk, water, etc.

Many bacteria and molds, as well as some yeasts, develop bodies which are known as spores. These are much more resistant to extremes of heat, drought, famine and chemicals than the ordinary or vegetative forms and serve to tide the organism over unfavorable periods which would have meant almost certain death and annihilation of the species. Whereas many bacilli in the vegetative stage are killed by ten minutes exposure to the temperature of 140 degrees Fahrenheit, some spores can withstand the temperature of boiling water for upward of sixteen hours. Many soil organisms possess spore stages, but, fortunately for man and beast, the number of disease producing bacteria which have this property is small.

Source of Plant Foods.

Between 97 and 98 per cent. of the weight of a growing plant is made from the carbon dioxide gas of the air and the water in the soil. The supply of these two substances is unlimited. The remaining 2 to 3 per cent., which constitutes the ash, is obtained largely from the soil, and the elements which compose this portion occur in limited quantities only. Year after year these mineral substances are appropriated by plants, and this constant drain for countless centuries would have exhausted the supply most certainly, were it not being replenished from some source. That this loss is restored is evidenced by the fact that our native plants continue to

grow for generation after generation on the same virgin soil without any apparent loss of vigor, the soil remaining as fertile as ever. Clearly, the problem for the scientific farmer is to ascertain the factors which have kept up the fertility of the virgin soil and apply them intelligently to cultivated land. By this means alone, can we hope to maintain undiminished the productiveness of our agricultural lands.

Number and Distribution of Bacteria in the Soil.

The prevailing idea of a soil is that it is a mixture of finely ground rock particles and organic matter capable of supporting plant life. Now, there was a time when this definition would fulfill the requirements, but, in the light of our present knowledge, it is altogether inadequate. It ignores absolutely the presence of millions upon millions of microscopic plants and animals whose presence is indispensable to the continued fertility of the soil. Let us remember, then, in passing, that a soil is not a dead mass of inert material, but rather that it is a teeming complex of living beings.

Of the three groups of plants which constitute the microbial flora of a soil, bacteria are considerably more abundant than either molds (fungi) or yeasts. Just as with the larger plants, with which we are more familiar, there are certain factors that affect the number of bacteria in a soil. Among the most important of these, may be mentioned moisture, air, organic matter, mineral foods, acidity, or alkalinity, toxic bodies and temperature. As a rule sandy soils contain the fewest microorganisms, relatively speaking, because of the deficiency of organic matter and the poor water holding power. However, if such soils are plowed deeply and a liberal amount of green material is thoroughly incorporated, the germ content may be increased considerably. An ordinary sandy soil will often contain less than 100,000 bacteria per gram (one twenty-eighth of an ounce), but after some attention has been given to its cultivation, the count may increase to several millions per gram.

In the rich loam soils, the numbers range from 1,000,000 to several millions and even into the billions, due to the high organic content which favors bacterial growth.

In the heavy, compact clay soils, such as our typical adobe, the germ count is considerably less, owing both to the poor aeration and absence of organic matter.

Under artificial conditions, such as are offered in a greenhouse, where the moisture, temperature, and food supply are especially favorable, it is not uncommon to find numbers ranging

around 100,000,000 per gram. The same is true of soil from sewage farms and of soil which has been partially sterilized either by disinfectants or heat in order to destroy certain animal forms that have been shown to feed upon bacteria. On the whole, it may be said that microorganisms are most abundant in the soils which are in the highest state of cultivation, and as a rule those soil conditions which are favorable to the growth of the higher plants are also well suited to the development of bacteria.

Distribution of Bacteria at Different Depths.

From what has been said already concerning the factors which favor the growth of soil bacteria, it would be natural to suppose that the organisms would be most abundant in the surface layers that receive manure and more thorough aeration through cultivation. This is exactly the case. Bacteria are most numerous in the first seven to eight inches of an arable soil; the germicidal action of the sunshine and the drying at the very surface reduce the numbers there somewhat. Below sixteen inches, the count falls off very rapidly, until at three feet, in the rainfall sections, it is said the soil is nearly sterile. The texture of the soil, however, makes considerable difference in the lower limit, since with a more open, loose structure, organisms are very apt to find their way to the lower levels. In irrigated regions, we know that bacteria frequently occur at depths greater than three feet, for it is not uncommon to find tubercles on alfalfa roots, produced by bacteria, eleven to twelve feet below the surface. Other bacteria have been found as deep as eight or ten feet in open soils where the drainage is good and where alfalfa roots have penetrated the earth to considerable depths. They have undoubtedly been carried down from the surface with the irrigating water and have followed the paths of alfalfa roots to the lower regions. In the more compact soils, we find the lower limit of microbial activity much nearer the surface since the conditions for aeration are poorer, and the filtering action of the finely divided soil tends to prevent a very extensive penetration even under a head of irrigating water.

THE DECOMPOSITION OF ORGANIC MATTER.

The Availability and Source of Plant Food.

The superior quality of well rotted manure, which has not been allowed to become worthless by leaching, as compared with the fresh product, is so well known to all experienced farmers that any comment on this point would be presumptuous. Again, when a heavy stand of alfalfa, red clover, or vetch is plowed under in a

green condition in the spring, the benefit from this is not apparent for three or four weeks. In other words, the plant food contained in the fresh stable manure or the green crop is locked up in some unavailable form which is of no use to growing plants until decomposition has set in. So far as the chemical elements present are concerned, they are essentially the same in both old and new manure, but the combinations are quite different after decay begins. Plants and animals, as well as the excrement from the latter, are made up of very complex compounds. Experience has taught us that it would be folly to attempt to grow a crop of oats on a barn floor by supplying the seed with fresh fish, alfalfa hay, or cotton-seed meal; but sow the oats in the ground, and let any of the above substances be incorporated with the soil, and in a reasonable length of time the effect of the fertilizer will be apparent. It would be equally absurd to think of raising a child, or a colt, or a calf, on a simple plant ration of nitrate of soda, superphosphate of lime and sulphate of potash with a little lime, magnesium, iron and sulphur for seasoning. Plants must have their food in a very simple form, while animals require theirs in a much more complex condition. Herein lies the explanation of the availability of plant food in thoroughly decomposed manures.

Were it not for microorganisms, there could be no such thing as decomposition, and every dead plant and animal would remain forever unchanged upon the earth. Not only would this globe soon become a rather unsightly dwelling place, but the energy locked up in these inert bodies would be an extravagant waste. Nature has provided wisely for this exigency, and has stocked the air, soil, and water with myriads of microscopic scavengers. (Plate III, 3, 4, 5, 6.) Although these are tiny plants, they can obtain their food from the dead bodies of plants and animals. In their ability to break these down and simplify them so that they can be of use to other plants, they rival our chemists with all of their splendid laboratory equipment.

THE TRANSFORMATION OF CARBON.

The chemical element, carbon, occurs in its purest form as the diamond and as graphite. We know it in a less pure state as coal, charcoal, coke and lamp black. When it is combined with hydrogen, it forms such compounds as illuminating gas, acetylene, and marsh gas. In the plant world it is found associated with oxygen and hydrogen in the form of sugar, starches, cellulose, woody tissue, fats, and waxes, and when nitrogen and sulphur are added to these three elements, the resulting compound contains the essentials

of protein. In fact, carbon enters into the composition of practically all plant and animal tissues. From this, it is clear that the demand for carbon must be very great, and in order to satisfy the demand, either the supply must be unlimited or the carbon is used over and over again. We shall find that the latter is the case. As pointed out elsewhere, plants obtain this element from the carbon dioxide of the air, of which about three parts in ten thousand are carbon dioxide. Animals secure their carbon from their foodstuffs, most of which come either directly or indirectly from plants. In the last analysis, it is plain to be seen that all of the carbon dioxide comes from the air. The supply of this gas is maintained practically constant in Nature through the oxidation of carbon and its compounds. Immense quantities of carbon dioxide are returned to the atmosphere during the burning of coal, wood, and gas. The carbon in these substances unites with oxygen to form carbon dioxide, the hydrogen combines with more oxygen to form water, and the mineral residue constitutes the ash. Many organic substances are eaten by animals, and, after serving their purpose, the carbon which enters into their composition is exhaled as carbon dioxide.

Another very important agency in setting free carbon dioxide is the work of micro-organisms. It was believed for a long time that the splitting up of complex substances during decay, accompanied by the liberation of different gases, was a purely chemical process, but it is very doubtful, in the light of our present-day knowledge, if this could take place without the intervention of bacteria, yeasts, and molds.

Sugars are among the first substances to be attacked by yeasts and molds in their search for food, and, as a rule, they are broken up into carbon dioxide and alcohol. This fermentation is perfectly familiar to anyone who has ever observed sweet cider changing into hard cider, or who has tasted jam or canned fruit which has "worked." The characteristic flavor in the latter case is due to the alcohol, and the frothing in the former is caused by the escaping carbon dioxide. The gas liberated in this way joins the supply in the air and once more it is ready to be taken up again by the leaves of some green plant and made into starch and sugar. The alcohol, likewise, passes into the air, under ordinary conditions, and ultimately is oxidized into carbon dioxide and water. Some bacteria, also, can accomplish the destruction of sugars. This may lead to the production of a number of alcohols, carbon dioxide, hydrogen, methane or marsh gas, and several different organic acids, among which may be mentioned butyric, lactic, acetic,

formic, and propionic. The first three of these are met with frequently around the farm, and when the place of their occurrence is mentioned, they will be recalled readily. Butyric acid gives the peculiar, rancid odor to butter and limberger cheese, and the bitter flavor to milk; lactic acid is the common acid of sour milk; the characteristic and pleasant acidity of cider vinegar is due to acetic acid. When such acids are set free in the soil, they act upon the mineral constituents and bring into solution certain compounds which are essential to plant growth. The acids are not apt to become very abundant in a well-drained and aerated soil, especially in Colorado, where there is so much calcium carbonate present with which they can combine. Furthermore, it has been shown that bacteria and molds can utilize the salts of certain organic acids such as acetates, propionates, butyrates, citrates, malates, succinates, tartrates, valerates and formates as a source of energy, and ultimately reduce them to carbonates, carbon dioxide and water. It is indeed a rare occurrence to find arable land in this state which is acid, although a few such have been noted by Dr. Headden. When this does happen, it is due largely to the presence of the so-called "humic acids" and acid humates, complex organic compounds about which we have but little definite knowledge. A liberal application of lime or ground limestone to soils in this condition usually alleviates the trouble in time. Blair states that the average acid soil of Florida requires 1,500 pounds of lime per acre to neutralize the acid to a depth of nine inches, yet it is not uncommon to find peat and muck lands where the acidity is many times this amount.

The alcohols produced are of considerable commercial importance as well as of scientific interest. They include ethyl or grain, propyl, butyl and iso-butyl alcohol.

The changes produced in starches are very similar to those met with in the sugars. However, before any carbon dioxide, alcohol, etc., can be set free, it is necessary to convert the starch into sugar. This is accomplished by a secretion from the microbial cells, called an enzyme, and the specific enzyme or ferment in this case is diastase. After this change is brought about, the fermentation and the products found are in all respects identical with those formed during the breaking down of sugars.

Cellulose, which constitutes a large per cent of the weight of woody tissue, and of which the cell walls of young plants are composed almost entirely, is very useful in maintaining the proper physical condition of a soil. Straw, cotton fiber and flax fiber are

common examples of this material. It is this compound in the spruces, aspens, and cottonwoods that makes them valuable for paper-making. When incorporated with the soil in the form of strawy manure or green crops, it decomposes slowly under the action of micro-organisms, keeps the land loose and open, and increases the water-holding capacity to a remarkable degree. Cellulose is a compound of carbon, hydrogen and oxygen, and is closely related to the sugars and starches in point of chemical composition. Certain bacteria of the soil, notably a group designated as *Amylobacter*, are known to feed upon it, and gradually to resolve it into carbon dioxide, hydrogen, methane and fatty acids. Here, again, the carbon dioxide is returned to the air. Some molds as well as bacteria can produce similar changes. However, the chemical reaction of the soil determines largely whether the decomposition will be brought about by bacteria or molds, since the former are active only in an alkaline environment, while the latter thrive best under acid conditions.

Anyone who has ever strolled or ridden through our mountain forests must have been impressed with the immensity of the timber waste. And yet is this waste? When we stop to think of the tremendous amount of energy and plant food which is being liberated through the decay and destruction of these fallen logs, the problem assumes quite a different light. The fertility of the mountain soils which wash into the gulches is too well known by every ranchman to need further comment. The fallen trees appear to remain unchanged for years, but with the passing of time, they become spongy and punky through some agency not clear at first, and slowly but surely the hard, woody trunk is converted into a soft, brittle mass which eventually crumbles into a brown powder. This soon becomes incorporated with the soil and furnishes food for other plants. The decomposition which takes place here is, for the most part, a destruction of cellulose compounds, and is carried on not only by micro-organisms but also by some of the large, fleshy fungi, familiar to us as toadstools. In fact, fungi, or molds, are largely responsible for the destruction of the woody tissue. The larger forms initiate the attack on the surface, and send their thread-like "rootlets," known as hyphae, beneath the bark and into the tissue. By means of enzymes and other chemical substances with which they are supplied, the wood is softened, and the way is paved for other fungi, and wood-eating insects. After the decay is well advanced, bacteria probably join hands with the other agents and hasten the final transformation. Thus, through the decay of timber, there are restored to the atmosphere carbon diox-

ide and water through the oxidation of carbonaceous material; and to the soil, carbonic acid, organic acids, organic matter and mineral substances.

As yet, nothing has been said about the destruction of fats, gums and waxes. Whether fats or waxes, bacteria, yeasts and molds, through the immediate action of enzymes, convert these fatty substances into glycerine and fatty acids, both of which may be oxidized finally into carbon dioxide and water.

Thus it is, that the carbonaceous materials contained in plant and animal tissues undergo decomposition at the hands of soil micro-organisms. The carbon of the complex compounds is transformed into carbon dioxide, which either passes into the air to replenish the atmospheric supply, or unites with water to form carbonic acid and ultimately soil carbonates.

TRANSFORMATION OF NITROGEN.

We come next to a consideration of soil nitrogen which, from the standpoint of conservation of plant food, is unquestionably the most important of all the elements. It is not that the supply of nitrogen is so limited, for approximately 79 per cent of the air consists of this gas, but rather, that it does not exist in any quantity in a form that plants can utilize. To be of value to vegetation, it must be present in the soil, either in the form of nitrates, or possibly as ammonium salts, but the stock of both of these is far from abundant.

The world's supply of commercial nitrate is derived almost exclusively from two sources—guano and Chile saltpeter; some nitrate of potash comes from the plains of the Ganges River in India. All of these are becoming exhausted rapidly, and already guano has ceased to be of any importance. While it is difficult to obtain any precise estimate of the amount of available saltpeter in the beds of Chile and Peru, South America, it is quite certain that if the present rate of consumption continues,—2,597,754 tons for the year 1910—it cannot last for any great length of time, some placing the limit at less than forty-five years. At \$50 or \$60 per ton, an application of two hundred to three hundred pounds of nitrate of soda per acre soon runs into money, and the commercial article, while rich in nitrate, is at the same time so expensive that the cost makes it almost prohibitive for the average farmer.

Dr. Headden informs me that our ordinary Colorado soils contain from .0037 to .0121 per cent (150-450 pounds per acre foot) of nitrates expressed as nitrate of soda, an amount far too small to permit of any wasteful methods in farming. From this it is quite clear that unless more attention is given by our agriculturists to

maintaining the soil-nitrogen by the application of manure or commercial fertilizer, it will be but a few years, relatively speaking, until our fields will be unproductive and worthless.

During their growth, green plants use nitrates to build up complex nitrogenous compounds which we call proteids, and the animals which feed upon them, convert these proteids into still different forms. Such substances as bone glue or gluten, mucus, gelatine, egg albumin, legumin and the fibrin of the blood are produced in this manner. A portion of the proteid appropriated by animals is retained throughout life, but a second part is consumed by the body to furnish heat and energy. However, while this is going on, the proteid is being broken to pieces. Much of the carbon is oxidized and exhaled as carbon dioxide, while the waste nitrogen is carried off by the kidneys as urea in the urine. In this way, the complex nitrogen molecule is considerably simplified, but not to such a degree that the resulting compounds are of any immediate benefit to plants. It has been estimated that the human race alone excretes 38,000 tons of urea daily, to which must be added the much greater amount voided by animals. In the light of this fact, does it not behoove all thrifty farmers to provide some means for conserving the liquid manure around the stables. Who knows how long such thoughtless waste is to go unpunished?

When these same plants and animals die, and they are returned to the soil, quantities of nitrogen remain locked up in the complex proteid compounds of their bodies. Neither they nor the urea, just mentioned, are of any use whatsoever to future plant generations unless their nitrogen can be converted into nitrates. Thanks to the presence of soil micro-organisms, a means has been provided by which the complex nitrogenous compounds in decaying organic matter can be converted into nitrates, and if the agriculturist will see to it that his land receives a reasonable amount of organic nitrogen from year to year, either as stable manure or green crops, and if he, himself, is equally diligent in the cultivation and irrigation of his soil, there need be no alarm over a nitrogen famine.

Ammonification.

In the transformation of the nitrogen of proteid bodies and their cleavage products we recognize three well defined stages. The first of these deals with the production of ammonia by bacteria from the complex organic matter; in the second, the ammonia nitrogen is converted into nitrous acid and nitrites, and in the third, the nitrites are changed into nitric acid and nitrates. The term "Ammonification" is applied to the first process while the combined

action of the last two is spoken of as "Nitrification." For the present, we shall confine our attention to ammonification.

Like the higher plants and animals, bacteria must have their food in a liquid condition before it can be assimilated. In order to facilitate the solution of the complex proteids from which the ammonifying bacteria derive their nourishment, certain digestive enzymes, similar to pepsin and trypsin in animals, are secreted by the tiny cells. As a result, the protein compounds are broken down first into albumoses and then peptones, the latter being soluble and consequently in a form which bacteria can use for food. From these peptones, amino acids are formed which themselves serve as food and ultimately give rise to ammonia and carbon dioxide. The enzyme which is responsible for this digestion is known as a proteolytic or peptonizing ferment since it is capable of changing proteids into peptones.

Bacteria which grow best in the absence of air (oxygen), known as anaerobes, are believed to play an important part in the early stages of putrefactive decomposition, and it is to their activities that we must charge most of the malodorous gas formation which accompanies decay.

Now, while the bacteria are busily engaged in decomposing nitrogenous material whereby their existence is maintained, a large number of substances of little or no value to them are split off from the complex nitrogen molecule. These are known as cleavage or by-products. Again, during the growth and metabolism of the micro-organisms themselves, various waste products are excreted from their cells. The disagreeable and peculiarly characteristic odors evolved during putrefaction and decay are often directly traceable to one or more of these secondary compounds. Thus we always associate the odor of rotten eggs with hydrogen sulphide, and the pineapple odor of old, rancid milk with butyric ether. The list of known substances which owe their origin to a fermentation of this nature is a long one, and in all probability is far from complete.

The decomposition of urea is of particular interest, since it is one of the most fertile sources of ammonia. This transformation is brought about by the enzyme *urcase*, which converts urea into ammonium carbonate, by simply adding a molecule of water, simple hydrolysis. Anyone who has been around a stable or a pile of fresh manure could not fail to have detected the strong odor of ammonia liberated from this substance. And right here is where a tremendous nitrogen loss occurs. Had the urine, from which the ammonia was formed, been absorbed by dry peat, saw dust or a

similar medium, a large part of the ammonia with its 82 per cent of nitrogen might have been saved instead of passing off into space. If absorbents are impractical, then the stables should be so constructed that the urine from the animals housed therein will drain into a pit or cistern prepared for this purpose. It should not be allowed to accumulate for any length of time, but should be sprayed or sprinkled upon the soil at once since there is considerable loss from standing. Since ammonium carbonate is a volatile substance, when manure is allowed to dry there is very great danger of the carbonate disintegrating and the ammonia escaping. For this reason, it is a poor policy, especially under our arid conditions, to spread manure on a field any length of time before it is to be plowed under. For the same reason, a manure pit is preferable to the ordinary compost heap. This should be located so that ditch water can be turned in from time to time and the proper moisture conditions maintained.

Throughout our study of the decay of nitrogenous material, we are chiefly concerned with the destiny of the nitrogen. This we have found is either set free as ammonia by the ammonifying bacteria during the process of ammonification or converted into ammonium carbonate by the uro-bacteria. If liberated as ammonia, it will either escape into the air or unite with carbonic acid, which is usually present during this fermentation, to make ammonium carbonate. In this form it is less apt to get away, if properly handled, and as such it will await the further changes to be initiated by the nitrifying organisms.

Soil bacteria, as a general thing, have greater endurance, and can withstand more severe treatment than other plants with which we are more familiar. Their growth and development in the soil are governed largely by conditions which are in man's control. They require about the same amount of moisture as other crops; a temperature of 82°—86° F. suits them; a well drained, aerated, thoroughly cultivated soil, abundantly supplied with organic matter favors their growth. They will not succeed well in acid land, and if such a condition exists, it should be corrected by liming.

Another factor which cannot be lost sight of is the beneficial as well as detrimental influence which our alkali salts may exert upon the micro-organisms. Lipman[†] of California, in his recent studies upon the effect of alkali salts upon ammonification has shown that ordinary salt, sodium chloride, and sodium sulphate

[†]Lipman, Chas. B., Toxic Effects of "Alkali Salts" in Soils on Soil Bacteria. I. Ammonification; Cent. f. Bakt. Abt. II., Bd. 32, No. 1-2, p. 58, 1911.

are toxic for ammonifying bacteria when added in small quantities to an alkali free soil. Sodium carbonate, on the contrary, exercises a stimulating effect when present in amounts equivalent to .1 per cent of the dry weight of the soil.

The ability to produce ammonia from organic matter is a function common to many soil bacteria. Marchal has reported seventeen different species which belong to this class. Among the more active ammonifiers may be mentioned *Bacillus mycoides*, *Bacillus proteus vulgaris*, *Bacillus mesentericus vulgatus*, *Bacillus subtilis*, *Bacillus janthinus*, *Bacillus coli communis*, *Bacillus megaterium*, *Bacillus fluorescens liquefaciens*, *Bacillus fluorescens putidus*, and *Sarcina lutea*.

A study of over thirty different Colorado Soils, representing the prevailing types to be found in the state, has shown them to possess exceptional ammonifying efficiency as compared with soils from other regions. The results obtained from six Colorado samples are given in the table which follows:

Table No. 1. *Ammonification in Colorado Soils.*

Source and Character.	Per cent. of nitrogen converted into ammonia in 7 days from 100 m. g. organic nitrogen as:			
	Cottonseed Meal.	Dried Blood.	Alfalfa Meal.	Flaxseed Meal.
Orchard; heavy adobe clay; niter	42.31	47.04	12.78	8.09
Orchard; clay loam; niter	51.98	47.98	15.30	1.12
Orchard; sandy loam; niter	47.10	52.64	13.69	.21
Orchard; sandy loam; normal	25.92	18.03	12.06	6.30
Beet field; gravelly clay; normal	28.02	39.79	2.83	5.26
Truck patch; silt loam; normal	21.09	25.08	.73	3.11

The results of this work indicate that both cottonseed meal and dried blood yield ammonia nitrogen readily under the action of our soil bacteria, and therefore they would be desirable materials to employ as commercial nitrogen fertilizers: the nitrogen of alfalfa meal appears to be about one-third as available as that of blood and cottonseed meal; while the nitrogen of flaxseed meal, relatively speaking, is almost unavailable.

A detailed account of our ammonification investigations has been published as Bulletin 184 of this Station.

Nitrification.

The change of ammonia and ammonia compounds in the soil into nitrates was looked upon for a long time as a purely chemical process. The transformation was explained as due to electrical discharges in the atmosphere, to natural combustion, or to the oxidation of organic matter in combination with calcium, magnesium, iron and manganese compounds. The reason for believing this was that nitrates are almost universally present in cultivated land combined with calcium or magnesium. In 1862, Pasteur expressed the belief that the formation of nitrates in the soil and in decaying organic matter might be due to biological activities. Muller, a few years later while studying the nitrites and nitrates in sewage and drinking water, came to look upon their presence as the work of micro-organisms. The exact nature of nitrification was not fully understood, however, until 1877 when Schlosing and Muntz demonstrated clearly that it was brought about by biological agents. Their classic experiments showed that when a dilute solution of ammonia is passed through a long tube filled with soil, nitrates appear in the resulting liquid in place of the ammonia contained in the original solution; in other words, contact with the soil had changed the ammonia to nitrate. Furthermore, if the soil in the tube is first sterilized by heat or chloroform or other germicides, and the ammonia solution is passed through subsequently, no change takes place. But if the sterilized soil is reactivated by inoculating it with a small quantity of fresh soil, its nitrifying power is again restored. It was quite clear from this that a close relation must exist between the life in the soil and the nitrifying process. Accordingly, bacteriologists set to work at once to discover, if possible, the specific micro-organisms which were responsible for the transformation. Years were spent in diligent search, but only negative results rewarded their labor. At last, in 1890, Winogradsky succeeded in isolating certain bacteria which were capable of producing nitrates from ammonia solutions. His investigations showed, further, that nitrification or the oxidation of ammonia into nitrates takes place in two separate steps, and that two distinct classes of bacteria are involved in these changes. The first stage is the oxidation of ammonia into nitrous acid and nitrites; the second, the oxidation of the nitrites into nitric acid and nitrates. Winogradsky designated the organisms which are responsible for the first step as nitrous or nitrite bacteria and called the two species *Nitrosomonas* and *Nitrosococcus*. The former represents varieties obtained from soil in Europe, Asia and Africa, while the latter,

those isolated from American and Australian samples. To the organism accomplishing the second step, known as the nitric or nitrate bacteria, he gave the name *Nitrobacter*.

In addition to these forms, Kaserer claims to have isolated an organism, *Bacillus nitrator*, which is capable of oxidizing ammonia directly to nitrate. Corroborative evidence upon this discovery has not been received as yet, and should it be confirmed, even then, the number of known nitrifying organisms is very small.

Nitrification, then, may be defined as the process by which ammonia compounds are changed to nitrites and nitrates by the action of nitrifying bacteria.

Inasmuch as nitrates in reasonable amounts are very desirable assets to all soils, it may be worth while to ascertain under what conditions nitrification proceeds most rapidly. In the first place, it is obvious that the soil must be supplied with adequate organic matter from which the ammonifying bacteria can produce ammonia. Since nitrification is an oxidizing process, the soil must be well aerated by thorough cultivation and proper drainage. One of the reasons that surface soil is more fertile than sub-soil is because the looser texture of the upper layers permits the easy penetration of atmospheric oxygen, a requirement for active nitrification. Conn states that, "about 65 per cent. of the total nitrification occurs in the upper twelve inches of soil, 30 per cent. more in the layers from twelve to thirty inches lower, and little or none below this." Sufficient moisture must be furnished for the growth of the bacteria, about the same as for ordinary crops. Nitrification takes place through a wide range of temperatures; feeble at 40° F.; marked at 55° F.; and best at 85° F. From this it will be observed that nitrification in the soil is going on most vigorously from July to October, a time of the year when the nitrates formed are of immense value to growing crops. The toxic substances excreted by the roots of higher plants are believed to be injurious to the nitrifying organisms, and therefore the urgent need of crop rotations. Acid soils prohibit nitrification, and if such conditions exist, they should be corrected by liming. There should be an ample supply of lime, magnesia and other basic materials to insure the prompt neutralization of the nitrous and nitric acid as well as any organic acids that may be formed. Excessive amounts of certain alkali salts may prove objectionable.

Among the numerous theories which have been advanced to account for the saltpeter beds of Chile and Peru, is the belief that the areas now occupied by these deposits were at one time arms of

the ocean, and with the gradual rising of the western coast, these bodies of water became inland seas, which could be replenished only at high tide. Quantities of sea-weed are supposed to have grown in these shallow basins, and after decomposing, their nitrogen was changed by nitrifying bacteria into nitrates. The high temperature, the lack of rainfall and the absence of an outlet all assisted in the concentration of the nitrate in these areas until the material which accumulated reached into the millions of tons.

Thus, through the agency of microorganisms by the processes of ammonification and nitrification, the unavailable nitrogen of decomposing organic matter is changed into nitrates and restored to the soil in a form which is indispensable to all plant life.

Nitrification* in Colorado soils is a topic to which we have given considerable attention during the past year because of its relation to the niter trouble which is discussed on page — of this paper. The results of the investigation indicate that this process goes on much more actively in our soils than in many soils from other parts of the United States. Work which is in progress at the present time may shed some light upon this point, but just now we cannot say whether it is due to peculiar soil conditions which are especially suited to the development of the nitrifying organisms, or possibly, to the presence of new species of bacteria which are exceptionally alert biochemically. This difference in nitrifying efficiency is illustrated very well in Table 2 which gives the net gain in nitrates made by Colorado and foreign soils when supplied with ammonia nitrogen in the form of ammonium sulphat and ammonium carbonate.

Table No. 2. Nitrification of Ammonium Sulphate and Ammonium Carbonate by Colorado and Foreign Soils.

Source and character of soil.	Net gain in nitrates per million parts of air-dried soil from 100 m. g. ammonia nitrogen as:	
	Ammonium sulphate.	Ammonium carbonate.
Colorado; wheat field; clay; normal.....	3258	2899
Colorado; peach orchard; heavy clay; normal	3681	2620
Colorado; apple orchard; heavy clay; niter	4302	2798

*The detailed account of this investigation has been published as Bulletin 193, Colo. Exp. Sta., "The Nitrifying Efficiency of Certain Colorado Soils," January, 1914.

Colorado; apple orchard; clay loam; niter	3541	1328
Colorado; apple orchard; sandy loam; niter	3629	2656
Colorado; apple orchard; clay; niter.....	3665	5081
Georgia; pine forest; gravelly loam.....	13	22
Oklahoma; cotton field; sandy loam.....	89	841
Washington; apple orchard; loam.....	133	841
Ohio; corn field; clay loam.....	266	620
California; vineyard; clay loam.....	513	loss
Virginia; apple orchard; heavy clay.....	620	1107
Kansas; sugar beet field; sandy loam.....	2833	3098

Duration of the experiment was six weeks at 28 degrees C.

Denitrification.

Denitrification may be defined as the partial or complete reduction of nitrates. It is the reverse of *nitrification*, and from an economic standpoint, is to be regarded as undesirable, since there is an actual loss of valuable nitrate. Whereas in nitrification, oxygen is added to ammonia and nitrites to form nitrite and nitrates respectively, in denitrification, the oxygen is taken away from the nitrates and they are reduced to nitrites, ammonia, and even to a point where free nitrogen is liberated, and oxides of nitrogen are formed. In a strictly scientific sense, denitrification applies only to the complete reduction of nitrates to gaseous nitrogen. The list of micro-organisms that can produce this phenomenon is a long one, and includes both bacteria and molds.

In soil and manure, most of these develop readily in the presence of air (aerobic), however, in the role of denitrifiers, they appear to be favored by the partial exclusion of air. A fine grained, close, compact soil, in other words a heavy soil, to which excessive quantities of fresh, strawy manure (50 to 60 tons per acre) have been added, presents the optimum conditions for bacterial denitrification. The free circulation of air is interfered with seriously in a soil of this character, and the growth of the denitrifying organisms is favored by the large amount of readily decomposable organic matter. Under such conditions, there may be a loss of nitrogen both from the soil and from the manure, since the bacteria, in all probability, will draw upon the nitrate for the necessary oxygen with which to liberate energy from the organic compounds. In this manure, the nitrates which we have been striving to restore to the land, and maintain there, may be changed from valuable plant food into an inert gas. On the whole, the danger from denitrification under field conditions has been greatly over-estimated, and

the farmer who applies from ten to twenty tons of well rotted manure per acre and who keeps his land well cultivated, need feel little concern from losses on this account. It is true, however, in truck gardens and greenhouses, where the ground is pretty well saturated, and where an abundance of organic material is used, a temporary loss of nitrates is sometimes experienced.

Those who have held that soil may suffer a loss of nitrates following the application of fresh manurs, if such is the fact, have explained the phenomenon by saying that vast numbers of denitrifying bacteria, which do the damage, are introduced with the manure. While the bacterial content of the soil is unquestionably increased by this addition, the reduction is probably due to an entirely different cause. The large amount of organic matter in the manure appears to be immediately responsible for the trouble by furnishing abundant food for the denitrifying organisms already in the soil. A mere increase in numbers would have little to do with stimulating activity, and furthermore, ordinary soils usually contain enough of these organisms to accomplish the reduction if adequate organic matter is supplied.

The manure heap presents an additional field for the study of nitrate reduction. There are so many factors that can influence microbial activities here that conflicting statements have arisen regarding the different changes supposed to take place. According to the best authorities, there may be some loss from denitrification in the surface portion where the air circulates more freely and where nitrates may have been formed by nitrification. Deeper down in the pile, it is a question whether there is any reduction since, except in very old manure, there are probably no nitrates present owing to the unfavorable conditions for nitrification and nitrate formation. On the other hand, in old, well rotted manure, where one might expect to find nitrates, there is not enough fresh organic matter to stimulate the denitrifying bacteria.

The Fixation of Atmospheric Nitrogen.

Separate and distinct from *Nitrification*, which has been described above, is the process of *Nitrogen Fixation*. The former deals entirely with the transformation of nitrogen already present in the soil, while the latter is concerned solely with the nitrogen of the atmosphere. Nitrification explains how the ammonia nitrogen which is obtained from protein decomposition is converted into nitrites and nitrates. Nitrogen fixation accounts for the addition and accumulation of nitrogen in the soil from the atmosphere.

The increase in fertility accruing to land fallowed for a number

of years is common knowledge to every experienced farmer. The available potash and phosphoric acid may have increased some, but the greatest difference observed is in the amount of nitrogen present. At the end of the fallow period, we should expect to find, and do find, more nitrate nitrogen due to nitrification, but far more important than this is the appreciable addition to the total nitrogen. Now the air with its 79 per cent. of nitrogen could easily supply this amount provided some means were at hand for transferring it from the atmosphere in a gaseous form to the soil in a combined form.

Many chemical theories were formulated and expounded during the third quarter of the nineteenth century to explain this phenomenon, but all failed when put to practical tests. For a long time it had been observed that plants of the legume family, represented by alfalfa, clover, peas, and beans, made good yields irrespective of the nitrogen in the soil. Not only was this true, but the soil itself was benefited by the legume, as was indicated by the crop that followed. This led to an analysis of the soil which showed very clearly that it actually contained more nitrogen after it had grown a crop of peas than before. In other words, the increase in nitrogen was, in some way, related to the life processes of the leguminous plant. Finally, the mystery was solved, and it was proven conclusively that the responsible agents were specific soil bacteria which both independently and when associated with legumes held the key to the great storehouse of atmospheric nitrogen, and that through their activities alone, this supply of nitrogen could become available for the higher plants.

We recognize two distinct types of activity and two different groups of bacteria in the fixation of atmospheric nitrogen. In the one, the micro-organism performs the work by itself; in the other, the combined action of germ cell and legume is required. The former is spoken of as *non-symbiotic* and the latter as *symbiotic fixation*.

Non-Symbiotic Fixation.

In the early experiments which were conducted by Berthelot in 1885, to determine the cause of the increase of nitrogen in fallow ground, two lots of soil were obtained from the same source. One portion was sterilized by heat to destroy all life, and the other was kept in its normal condition. Both samples were exposed side by side to the same conditions and both were treated as nearly alike as possible. After a given length of time had elapsed, the total nitrogen was determined in each, and it was found that the heated

portion had remained practically unchanged, while the other showed a decided gain. This proved beyond a doubt that the nitrogen increase in the fallow ground was in some way intimately associated with the life of the soil microorganisms. Winogradsky, in 1892, succeeded in isolating an organism, *Clostridium pastorianum*, which was capable of forming nitrogen compounds, under anaerobic conditions, in the culture medium which contained no nitrogen. A very valuable addition to our knowledge of the subject was made when Beijerinck, in 1901, reported his discovery of a group of large, aerobic bacteria capable of building up protein compounds from atmospheric nitrogen. To this group, he gave the name *Azotobacter*. Two species were described by him, *Azotobacter chroococcum* and *Azotobacter agilis*, and since then five or six others have been added to the list. Both the anaerobic and the aerobic forms are widely distributed in cultivated soils. In addition to these, a slight fixation has been secured with several of the ordinary soil bacteria but not enough to be of any economic importance.

In this connection may be mentioned the commercial preparation "Alinit," a pure culture of *Bacillus ellenbachensis*, which was exploited a number of years ago in Germany as a panacea for worn out land. It was to be used for inoculating soil and by virtue of the nitrogen fixing bacteria which it contained, wonderful gains in nitrogen were to be expected. Like many such phenomenal discoveries, it failed to accomplish the extravagant claims made for it, and it was soon forgotten save for its record in the annals of science.

Several attempts have been made to inoculate soil with pure cultures of *Azotobacter* but with little success. As with Alinit, the failure to secure satisfactory results have been due in a large measure to unfavorable soil conditions, namely, acid soils, insufficient moisture, lack of basic carbonates, and mineral salts.

While the mechanism of nitrogen fixation within the bacterial cell is not clearly understood, it is believed that the *Azotobacter* cell utilizes the nitrogen of the atmosphere to build up protein compounds. These in turn undergo decomposition, and through the action of the ammonifying and nitrifying organisms, they are converted into nitrates.

It is believed that during the process of fixation, *Azotobacter* derives the energy for this function from the organic material present, and the results of certain experiments have shown that the amount of nitrogen taken up bears a definite relation to the quantity of carbonaceous material consumed. From this it follows

that an abundant supply of organic matter in the soil is one of the requirements for active nitrogen fixation.

A careful study of some thirty Colorado soils was made by the writer over three years ago, for the purpose of learning whether there was any relation between the high nitrate content of these soils and soil bacteria. The samples collected represented orchard land, raw land, beet, alfalfa, and grain fields, and truck gardens. It was suspected that possibly *Azotobacter*, favored particularly by the alkaline reaction of the soil, was fixing immense amounts of nitrogen as protein, which was subsequently converted into nitrates by ammonification and nitrification. This question is of tremendous economic importance since the amount of niter in some localities is approaching 100 tons per acre foot, and all kinds of vegetation are dying as a result of the accumulation. In describing the serious condition brought about by this trouble, I can do no better than to restate what I have said on this subject in a former bulletin.

"Somewhat over a year ago, Dr. Headden called my attention to the extremely large quantities of nitrates present in certain Colorado soils, stating at the time that these nitrates were frequently associated with a brown discoloration of the soil, and that this color was often confined to well defined areas varying in size from three feet in diameter to an acre or more; furthermore, that these so called "brown spots" were not fixed, inert quantities related to some recognized geological formation, but that they were alive, and in the process of making as evidenced not only by the rapid progress with which the then existing spots were spreading, but also by the almost continual appearance of new spots both in old and new localities.

Dr. Headden has been studying our alkali soils and drainage waters for the past sixteen years, and he tells me that complaints of "brown spots on which nothing will grow" have been common, but more so during the past five years; reports have been received from the cantaloupe growers that their melons are deteriorating in quality without any assignable cause; truck gardens, alfalfa, oat, barley and sugar beet fields have been developing barren patches where a uniform stand was always obtained in former years; in some parts of the state, the sugar content of the sugar beets as well as the purity and tonnage have fallen off until it is a ponderous question with the farmers and sugar factories whether the growing of sugar beets in those localities is any longer a profitable industry; but equally serious, if not even more so than any of these, is the destruction which is being wrought in some of the apple orchards of Colorado.

Newly set trees, trees that have just come into bearing, and trees that are fifteen to twenty-five years old, in fact, trees of all ages, seem to suffer alike. It is not an isolated tree here and there that has died, but thousands, representing many acres of orchards in widely separated districts, have perished during the past two seasons.

When one is brought face to face with facts of such tremendous economic importance as these, he can scarcely fail to be impressed with the deplorable condition of affairs, and is forced to the position that something out of the ordinary is taking place, and that it is not without a cause.

With reference to the occurrence and distribution of the nitre areas, Dr. Headden gives the following in Bulletin 155 of this station:

'This trouble was not confined to any one section, but was common to several sections of the state. While it, in all probability, depends upon soil conditions, these conditions are met with in so many places that it is necessary to consider the condition rather than the soil itself. It sometimes occurred in light and sandy loams, and sometimes in clayey soils. It is sometimes in comparatively low lying lands, again in the low lying portions of higher lands, and again on the hillsides. The road side, a ditch bank, and the cultivated fields represent the range of places in which this thing may reveal itself. There is one thing common in all of its occurrences, namely, a brown color in the surface soil. This color is less marked in the sandy soils than in the so-called adobe soils. Perhaps this is due to the presence of the deliquescent salts on the surface of the adobe soils, or more probably to the color of azotobacter films.'

'We find the nitrates present in soils, where there is a great deal of moisture, but in places where there is too much water, the nitre does not appear. In little valleys and saucer shaped depressions in which the lower portions are too wet, there is no visible alkali, then follows a zone where white alkali abounds and above this the nitre is formed. I do not mean to say that there may not be nitre mixed with the white alkali, but that the nitre in such cases appears on higher ground than that on which the white alkali usually appears. Furthermore, it is not intended that anyone shall infer that it is only in valleys and depressions that the nitre occurs.'

In driving through those districts which are suffering with this trouble, the most striking feature to one not conversant with the symptoms is the brownish, black and, to all appearances, wet condition of the soil. This can be seen along both sides of the travelled road, and often extends to the irrigating ditch, or fence on either

side, and into the adjoining fields. I think of nothing which describes the color better than the appearance of soil where crude oil has been spilled, as is done frequently in orchards where oil pots have been used in heating, or, if you please, where the roads have been sprinkled with oil. Considerable disappointment is experienced, however, when this blackened surface soil is examined for it is often found to be a dry crust, rather than a wet one, one-fourth to one-half inch in thickness, underlaid with one or two inches of material of a very mealy character, beneath which the soil looks like any other soil. Sometimes the surface is so moist as to be slippery, due, probably, to the presence of quantities of deliquescent salts. As one walks over a field in this condition and breaks through the hard crust, the sensation experienced has been likened to walking on corn meal or ashes.

Concerning the condition of the soil met with under the mealy layer, I can not go into details since Dr. Headden has treated this phase of the question very fully and completely in his publications, suffice it to say that free water is seldom found nearer to the surface than five feet, and in most cases the soil is in what would be considered a nice moist condition; again in the heavier lands, we may expect and do find them rather sticky near the surface and of a gumbo character as the water plane is approached.

The brown color often appears on the banks of the irrigating ditches, eight to ten inches above the level of the water, and along the upper edge of the irrigation furrows. Extending lengthwise of these, it manifests itself a few days after irrigation as broad bands of pigment which might easily be mistaken for manure stains, so far as color is concerned, especially if the field or orchard had been fertilized recently. It is not uncommon to find large tracts of land where the nitrates have become so abundant as to be deleterious to the crops, yet where no discoloration is apparent on the surface. It is difficult to say in such instances whether no color is being produced or whether it is developing so gradually and uniformly that it can not be detected readily.

The economic outlook of this problem is, indeed, a serious one. Bushels of wheat have been planted on heavy nitre soils, and if it germinated at all, only a very small percentage ever came through the ground. Oats and barley have suffered the same fate. Corn has germinated in some fields, and made a sickly, yellow growth of six inches to a foot and then died. Sugar beets, if they grew at all, have gone to tops, while the roots have taken on all sorts of abnormal, irregular shapes, typical "tub-beets," to say nothing of the inferior

quality of the beet from the sugar standpoint. Dr. Headden has collected a great deal of data on this point which will be presented by him in due time. The money loss to the farmers in seed alone has amounted to thousands of dollars. But the orchardist has been unquestionably the heaviest loser, for not only has he been deprived of the crop for the current season, but he has also lost the trees upon which he is dependent for future crops, at least we have yet to see a single tree which has shown any indication of recovery. Added to this and worse than all, perhaps, is the utter worthless and hopeless condition of his soil for agricultural purposes. The apple, cherry, apricot and plum, all appear to suffer about equally, while the pear and peach, thus far, have exhibited marked resistance, the peach having been observed to suffer least of all.

The symptoms of excessive nitre in the soil as manifested in apple trees are so characteristic that it may be well to describe them briefly in passing. The first indication is the firing or burning of the leaves along the margins, beginning at the apex, extending rapidly along the edge, inward toward the midrib and downward toward the base until the entire leaf has turned brown. There is no occasion for anyone who is familiar with the yellowing of foliage due to lack of proper drainage to confuse this with the nitre burning, for the appearance of the leaves in the two cases is entirely distinct. Whole trees have been known to undergo this transformation in less than three weeks time. In fact, Dr. Headen reports having killed a four-year-old tree in an experimental orchard in four days by applying twenty pounds of nitrate of soda around the roots and then irrigating at once to bring the nitre into solution. In reference to the behavior of this tree, he says, "The effects were in all respects similar to those produced in other orchards" under natural conditions. If the burning of the leaves occurs early in the season, the trees will often exert a feeble effort to put forth a second crop of leaves. These are usually small, whitish leaves and inclined to be rather pubescent. Such trees, laden with one-third to one-half grown apples, seldom mature any fruit, and in all probability will be dead by spring. If the attack comes late in August or September, the chances are that the fruit will mature, but it will be undersized and of poor quality; no new leaves will be expected to appear and the old ones will cling to the twigs late into the fall. The following spring, it is very likely that an attempt will be made at leafing out but as stated above the leaves will be small, yellowish-white and few in number, and by the middle of the season the tree will be dead.

Before proceeding further, I wish to make it perfectly clear that what I have said is not to be interpreted as applying to all of our

arable land or to more than a very small percentage. While the matter is eminently serious, it by no means justifies the position that our agricultural interests as a whole are in jeopardy."

A full account of this investigation has been published already (1), the results of which show that *Azotobacter chroococcum* is present in most of the soils examined and that the characteristic dark brown color referred to above is due, in a large part, to the pigment produced by *Azotobacter chroococcum*.

In view of the results which we have obtained in our studies of non-symbiotic nitrogen fixation in Colorado soils, together with those cited in connection with ammonification and nitrification, we feel reasonably safe in asserting that the excessive nitrates which we find are the result of bacterial action.

In order that the reader can have a more definite idea of the amount of nitrates which have been found in some of these once arable soils, I am giving below in Table No. 3, a few figures on this point which have been furnished to me by Dr. Headden.

By way of comparison, I may say that an average amount of nitrate, expressed as nitrate of soda, for our cultivated fields is from .0037 to .0121 per cent., 150 to 450 pounds per acre-foot.

Table No. 3. *Nitrates in certain nitre soils.*

Source	Material examined	Percent water soluble	Percent nitrates in water soluble	Percent nitrates in air dried soil
Black spot in barley field..	Surface soil two inches	13.4	41.859	5.628
Young orchard	Surface soil.....	22.466	29.114	6.54
	Surface soil two inches	8.23	8.173	.673
Young orchard	inches	8.23	8.173	.673
Alfalfa field.....	Top soil five inches	7.78	33.06	2.571
	Surface soil two inches	5.42	50.221	2.722
Oat field.....	inches	5.42	50.221	2.722
Orchard	Top soil 12 inches	6.51	43.57	2.837
Corn and rye...	Surface soil	4.67	7.352	.342
Old orchard.....	Surface soil	6.65	5.746	.382

These figures may mean more when I say that one of the above samples, which carried 2.873 per cent. of nitrates in the

(1) Bacteriological Studies of the Fixation of Nitrogen in Certain Colorado Soils, Bulletin 179, Colorado Experiment Station, 1911.

surface foot, contained nitrates corresponding to 113,480 pounds, or 56.74 tons per acre foot; in another sample, taken to a depth of five inches, the area involved being about eight acres, sodic nitrate corresponding to 344,000 pounds or 172 tons was found in the surface five inches; in the top four inches of another eight acre tract, the equivalent of 189,971 pounds or 95 tons was found.

With such quantities of niter in the soil as these figures indicate, it seems hardly necessary to look elsewhere for an explanation of the death of our trees and crops.

Our laboratory studies and field observations have led us to believe that a number of factors are concerned in bringing about this sad condition of certain Colorado soils. There seems to be little doubt that in many cases the trouble has been aggravated by the too liberal use of irrigating water. To over-irrigate seems to be a common fault with many of our agriculturists. *Azotobacter* thrives best when the soil contains from 15 to 18 per cent. of moisture, practically the same as that required by growing crops. It does not succeed well where the ground is too wet or badly seeped; however, a constant supply of moisture favors its development. Continued, clean cultivation stimulates the activity of the organisms both by aerating the soil, and by raising the soil temperature through the free access of the sun's rays. The neutral or alkaline reaction of Colorado soils, due to the large amounts of carbonate, is unquestionably the limiting factor which makes our soils superior to those of the East and Middle West in promoting the growth of *Azotobacter*.

The one objection that has been raised to the *Azotobacter* theory, as an explanation of the excessive nitrogen, is the small amount of organic matter in our soils from which to derive the energy necessary for such a large amount of fixation. In a measure, this is true, if we understand the term *organic matter* in the ordinarily accepted sense; our soils are not rich in this constituent. It has been demonstrated that some of the simpler green plants that grow in the soil and in water, known as algae, can furnish *Azotobacter* with this energy. Following this clue, we have undertaken a systematic study of the algae which occur in the niter soils, and I am glad to be able to state at this time that our search has been richly rewarded. Professor Robbins, who has had charge of this phase of the investigation*, has found some twenty-four different species of blue-green algae, occurring in

*Bul. 184, Part II, Colo. Exp. Station, "Algae in Some Colorado Soils," June, 1912.

relatively large numbers, in these areas. If it is true that algae and organic matter are equally valuable as sources of energy, then there should be no question about energy for Colorado Azotobacter. However, in regard to algae and organic matter being the sole sources of energy for these organisms, we are open to conviction.

Symbiotic Fixation of Atmospheric Nitrogen.

The liberal use of leguminous crops such as red clover and alfalfa in the role of green manures has come to be recognized today as one of the most important steps toward placing our agriculture upon a modern, scientific basis. There is not a practical farmer but who acknowledges the inestimable value of plowing under a heavy alfalfa sod for potato land. If he is a successful man, he does not, or at least he should not, follow wheat with wheat, but seeds his field to alfalfa or some other leguminous plant which he has found by experience will make up in some mysterious way for the deficiency in soil fertility caused by the preceding crop. This is the principle upon which the rotation of crops is based.

If the Leguminosae possess such restorative properties, there must be some peculiarity in their nature and habits to account for this remarkable power. From the earliest days of agriculture, it has been recognized that this family of plants has a decidedly beneficial effect upon the soil. Almost nineteen hundred years ago, it was observed and noted by such men as Pliny the Elder that, "The bean ranks first among the legumes. It fertilizes the ground in which it has been growing as well as any manure. The lupine enriches the soil of the field or vineyard as well as the very best manure. The vetch, too, enriches the soil and requires no attention in its culture." Varro, in *De Re Rustica*, I, 23, writes, "Legumes should be sown in light soils; indeed they are planted not so much for their own crop as for the following crop, since when they are cut and kept upon the ground they make the soil better. Thus the lupine is wont to serve as a manure where the soil is rather thin and poor." The early writings on agriculture are full of just such references to the importance and necessity of including some leguminous crop in the regular rotation. Naturally, the explanations for this conservation of fertility were many and various, but the one which received most favor was the belief that the root system of these plants was much more extensive than that of grains and root crops, and in consequence brought up plant food from greater depths, which was not only available for the legume, but also served for subsequent crops. Thaer in 1809 ventured the

statement, without any experimental evidence, that the cultivation of leguminous plants might improve the soil by taking up nutrient material from the air and depositing it in the soil by means of the roots and stubble. In the light of our present knowledge, this was, indeed, a remarkable conjecture. Some years later John showed that there was not only an increase in humus following a leguminous crop but also a definite increase in the nitrogen content. This discovery led to numerous attempts to explain the source of the added nitrogen. The most common theory advanced for this phenomenon was the absorption of nitrogen gas by the plants through the leaves, but the classic experiments of Boussingault, in 1854, together with those of Gilbert, Lawes and Pugh showed the fallacy of any such idea. Nothing which approached an explanation of the question was reached until 1886 when Hellriegel and Willfarth demonstrated that the source of nitrogen for this class of plants was unquestionably the air, and that when such plants were grown in nitrogen free soil, the growth always took place after the formation of nodules or swellings on the roots, and therefore there must be some definite relation between nitrogen fixation and root nodules. The results obtained by these two men were later substantiated by the investigations of others and a solution of the long unsolved problem was promised.

This discovery cleared up quite a big part of the mystery connected with the question, and investigators at once turned their attention to finding a possible cause for the formation of these galls or nodules. Insect bites, worm bites and pathogenic fungi were resurrected to explain these hypertrophied roots. Some considered them fungus growths similar to sclerotia; others looked upon them as modified lenticels, undeveloped buds and spongy roots. As early as 1860, Woronin described these structures in detail and said he believed they were caused by vibrio-like organisms which he had discovered within the nodule, but he carried his work no farther. Later observers also found these so-called bacteroids but paid little attention to their significance. Accounts of their work indicate that they were seeking larger game.

In 1879, Frank showed that these root tubercles were absent on plants grown in sterilized soil, and that they were not mere reserve storehouses for protein substances, but stood in direct causal relation to the fixation of atmospheric nitrogen. Although Woronin almost thirty years before had discovered that these enlargements were filled with micro-organisms, their true bacterial nature was not definitely established until 1888 when Beijerinck

isolated and grew them upon an artificial culture medium composed of a decoction of pea leaves, gelatin, asparagin and saccharose. To the causal organism he gave the name *Bacillus radicicola*.

Following close upon the discovery of the relation of nodule bacteria to nitrogen fixation in the Leguminosae came the idea of utilizing these bacteria commercially. Experiments had shown that where soils were deficient in these germs, they could be made much more productive if inoculated with a soil known to contain an abundance of the coveted organisms. Accordingly, there have appeared on the market in recent years pure cultures of these bacteria under the trade names of "Nitragin," "Nitro-Culture," etc. It was intended primarily to use this material on the seed, but it could be employed to inoculate the soil if desired. Columns of print have been used to exploit these preparations which are offered for sale at the modest price of \$2.50 for material sufficient for one acre.

The first cultures that were distributed in this country were put up in a dry condition on small tufts of absorbent cotton. From these, the purchaser prepared his own liquid culture with which to inoculate the seed by adding the dried material to a certain nutrient solution, the ingredients of which accompanied the cotton. This method failed for two reasons: first, the bacteria could not withstand the drying on the cotton; and second, the farmer possessed neither the facilities nor the technical skill necessary to grow the liquid cultures and keep them pure. The outcome was cultures of doubtful purity and virulence, with a glaring lack of uniformity and agreement in the results obtained by using them. This difficulty has been overcome in a large measure by the preparation and sale of concentrated liquid cultures and cultures put up on a solid medium, so that all the purchaser is required to do now is to shake up or mix the cultures with a stipulated amount of clean water, and use this at once to inoculate the seed. Kellerman, of the U. S. Department of Agriculture, reports successful inoculations in 76 per cent. of his field tests with the liquid culture, and Edwards, of the Guelph, Ontario Experiment Station, has secured positive benefits in 65 per cent. of his trials with the solid form.

As the question stands at present in the United States, practical experiments have shown that the use of pure cultures is attended with much uncertainty. Where the soil is well adapted to a leguminous crop, pure culture inoculations have succeeded very well, but where the soil conditions are adverse, the simplest,

surest and most economical method of inoculation is by means of soil collected from a locality where the desired bacteria are abundant as indicated by the nodules on the roots of plants. One to two hundred pounds of such soil per acre scattered over the field will almost always produce a satisfactory inoculation, other things being favorable.

Although the nodule forming bacteria are cross-inoculable to a certain extent on different legumes, a higher per cent of successful inoculations has been obtained when the culture employed has been isolated from the same species as the legume to be inoculated. For this reason, when ordering either cultures or soil, the specific legume for which the material is desired should be stated. For example, an alfalfa culture should be purchased for alfalfa, and not a bean or a cow pea culture; the same applies to soils.

In using soil for inoculating land, the danger of introducing obnoxious weeds and plant diseases must not be overlooked. In this connection may be mentioned the recent discovery that the organism of crown-gall, *Pseudomonas tumefaciens*, is widely distributed in cultivated soils, and that its attacks are not confined to fruit trees only, but that they extend to the sugar beet, salsify, tomatoes, and many other plants, including the legumes.

The chemical reaction of the soil has been shown to be a limiting factor to successful inoculation, neutral and alkaline soils being preferred. Another investigator has observed that seeds in swelling, previous to germination, secrete a substance which tends to prevent the formation of nodules. The character of the soil, the sub-soil, and the drainage are additional factors which must be taken into consideration.

CHANGES IN MINERAL SUBSTANCES.

In addition to the carbon and nitrogen which enter into the composition of plant tissues, there are several other elements equally indispensable to their growth. Phosphorus, potassium, sulfur, calcium, magnesium, iron and silicon, all share in the make-up of green plants. Inasmuch as these elements occur in all soils, more abundantly in some than in others, we have given ourselves little concern over the time when they shall cease to be plentiful. Already, the potash, phosphoric acid, and lime (calcium) have become practically exhausted in some soils, and they are being supplied in the form of commercial fertilizers as superphosphate of lime, sulphate of potash and limestone respectively. In some soils, it is not so much a question of the supply being depleted as it is a matter of convert-

ing the material at hand into forms which plants can use. It is this phase of the question which will be taken up in the next few pages.

Phosphorus.

Soil phosphates as they occur in Nature, are but slightly soluble in water, and during the growing season, when the demand is heavy, they often fail to go into solution rapidly enough to satisfy the needs of plants. In soils where the phosphoric acid is low, the deficiency is usually made up by applying some form of commercial fertilizer known to contain a considerable quantity of phosphorus. For this purpose, either superphosphate of lime, ground bone, phosphate rock, or Thomas slag may be used. With the exception of the superphosphate, the phosphorus in the other materials is in an insoluble form, and therefore of no immediate value to plants. As has been noted before, during the decomposition of organic matter, a number of organic acids are formed by soil micro-organisms. It will be remembered, also, that quantities of carbon dioxide are liberated during sugar and starch fermentation, and that this unites with water to form carbonic acid. Now, while all of these acids are weak, relatively speaking, they nevertheless play a very prominent part in bringing the insoluble phosphates into solution.

Some time ago the writer conducted a series of experiments to determine the value of these acids as solvents for the phosphate of ground bone and phosphate rock. The results of this work* indicated that when bonemeal was added to milk, and the milk was allowed to sour, 25.31 per cent. of the phosphate was made soluble by the lactic acid which developed in the milk. Again, when ground bone was added to beer wort which was undergoing both alcoholic and acetic fermentation, 53.55 per cent of the phosphate became soluble in 15 days. Employing dilute lactic acid, 0.1 per cent., 3.13 per cent. of the bone phosphate was rendered soluble in five days; while the same strength of acetic acid gave 4.12 per cent. These figures will suffice to show that the dilute organic acids, though weak, are nevertheless potent factors in bringing insoluble compounds into solution. Other experiments conducted at the same time lead us to believe that acids are not the sole solvents, for, under favorable conditions, certain soil bacteria, independent of acid formation, are capable of converting small quantities of insoluble phosphates into a soluble form. In one case, 1.19 per cent. of the insoluble phosphate

* The Solvent Action of Soil Bacteria upon the Insoluble Phosphate of Raw Bone Meal and Natural Raw Rock Phosphate. Sackett, Patten and Brown. Special Bulletin No. 43, Michigan Experiment Station. 1908.

of bone was made available; in another, .46 per cent of the phosphate of phosphate rock was changed into a soluble form. In neither case was any acid present. Similar phenomena have been reported by Stoklasa, and Koch and Kroeber. Although our data on this line of investigation is very meager, there is reason for believing that the conversion is brought about through enzyme action.

The marked deficiency of phosphoric acid in acid soils is further evidence that soil acids are directly concerned in liberating phosphoric acid from its mineral compounds. At the same time that soil micro-organisms are breaking down organic substances and liberating acids to dissolve the phosphate, they, themselves, are appropriating small quantities of phosphoric acid for their own use. Like the higher plants, we find phosphates present in their tiny cells in very appreciable quantities. The ash of bacteria and fungi frequently contains more than 50 per cent. of phosphate.

Plants obtain their phosphorus from the soil as soluble phosphate, and animals, in turn, feed upon these plants. Both contain phosphates in their tissues, but in forms too complex to be of service to future generations of plants until they are simplified. This is accomplished in time by bacterial decomposition, and the complex phosphates are again returned to the soil. Thus, the phosphorus cycle in Nature is completed.

Potassium.

The principal source of potassium in our soils is the potash feldspar which occurs in quantity in the country rock. Through countless ages, these great masses of granite have been crumbling and disintegrating, due to the action of heat and cold, freezing and thawing, wind and rain, until today the finely divided particles constitute a large part of the soil. Orthoclase, the potash bearing feldspar, is a silicate of potash and alumina. However, under the agencies of decomposition, its identity is lost in the formation of potash zeolites, hydrated silicates of alumina and potash. It is in this form that the potash is found in the soil. Such a compound is far too complex to be of any immediate benefit to plant life unless some easy means of getting it into solution can be found. Here, as with phosphorus, the organic acids and carbon dioxide produced during the decomposition of organic material by soil bacteria attack the potash zeolites and set free the potassium, as potassium carbonate, which is readily soluble in water. The nitric acid formed during nitrification and the sulphuric acid resulting from the oxidation of hydrogen sulphide together with various organic acids may also combine with potassium to form potassium nitrate, potassium sul-

phate and a number of organic salts of potassium. Once more we see that the micro-organisms of the soil are the responsible agents in restoring potassium to the soil in a form which plants can use.

Sulphur.

In our arid soils, sulphur abounds in calcium sulphate as gypsum, and in sodium sulphate and magnesium sulphate, two of our common white alkalis.

When protein compounds undergo decomposition in the absence of oxygen, quantities of hydrogen sulphide are evolved. This gives rise to the disagreeable odors so characteristic of putrefaction. The oxidation of the hydrogen sulphide, liberated in this manner, furnishes energy to a group of organisms known as the "sulphur bacteria." As a result of this oxidation, free sulphur is produced which may often be seen within the bodies of the bacteria as minute red granules. The sulphur may be further oxidized to sulphur dioxide which later may be changed to sulphuric acid in the presence of oxygen and water. Such a thing as a free sulphuric acid in our soil, however, would be a rare occurrence, for it would combine immediately with a lime or magnesium base to form calcium or magnesium sulphate. Plants obtain the sulphur for their proteids from some of the soluble sulfates and animals feed upon these plants. When plants and animals die, the sulphur present in their tissues is restored to the soil through the combined action of decomposing and sulphur bacteria.

Iron.

Among the larger bacteria, we find certain groups which are characterized by a deposit of iron rust or iron oxide in the sheath surrounding the cells. Attention has been called to these, particularly because of the trouble which they have caused in municipal water supplies by plugging the pipes with their growth. These organisms are said to possess the power of precipitating iron oxide out of a dilute solution of iron salts; and through the exercise of this function, great masses of a slimy, gelatinous, rusty growth are produced in the water mains which give no end of annoyance to those who have charge of the distribution of such water.

In considering the relation of bacteria to the iron compounds, the deposits of bog iron in swamps and wet places are of considerable interest since it was believed at one time that they owed their origin to the reduction of iron sulphate by bacteria. Later authorities, however, claim that the precipitation of the iron oxide is a mechanical rather than a chemical process, but whether chemical or

mechanical, it is a significant fact that bacteria are intimately associated with the phenomenon.

Soil bacteria, like other plants, need a little iron for their growth. Lipman and Koch have noted that this is particularly true for the nitrogen fixing organisms.

Calcium (Lime) and Magnesium.

So far as furnishing favorable conditions for the growth and activity of micro-organisms, the arable soils of Colorado offer all that could be desired. The one thing, above all others, which makes them especially well adapted to the development of soil bacteria is their neutral or alkaline reaction. This is made possible by the presence of quantities of calcium and magnesium compounds. Frequent reference has been made already to the damage which may result to an otherwise fertile soil by allowing it to become acid. When this condition exists practically all bacteriological processes cease. The principal function of the calcium and magnesium carbonates is to neutralize the various acids formed during protein-decomposition; to combine with the nitric acid resulting from nitrification by which calcium and magnesium nitrates are formed; and to unite with the sulphuric acid set free by the so-called "sulphur bacteria" to form calcium and magnesium sulphates. Thus, the soil acids, which if allowed to accumulate might prove detrimental, are neutralized, and all injury to the soil flora from this cause is prevented.

Although our soils at present are rich in calcium carbonate and sulphate, there is nevertheless a large annual loss due to the appreciable amounts which are carried away in the drain waters. We have seen that carbon dioxide is generated in quantity during the fermentation of sugars, starches, etc. When this gas is dissolved in water it constitutes carbonic acid, which has a marked solvent action upon carbonate of calcium. Through this agency, immense caves have been formed in the limestone regions of the United States, and tons of rock are transported annually to the ocean in solution as the acid carbonate of lime. A small portion of this is utilized by the various shellfish in the construction of their shells; the remainder goes to form the limestone deposits of future generations.

PLATE I



1.



2.



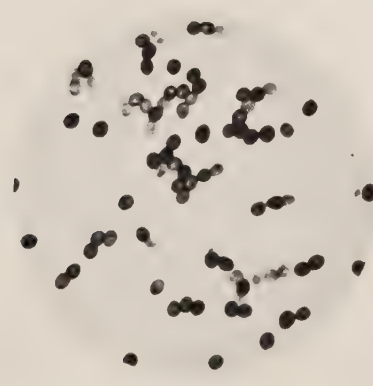
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6.

PLATE II



1.



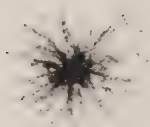
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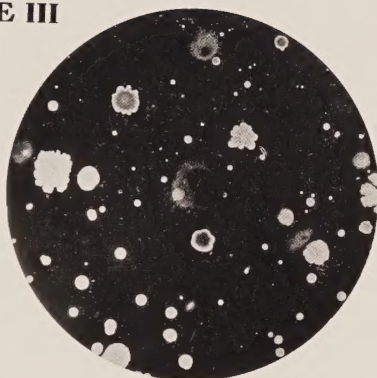


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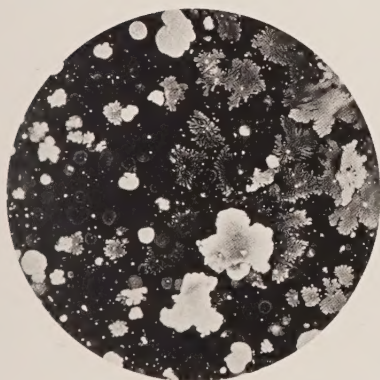
PLATE III



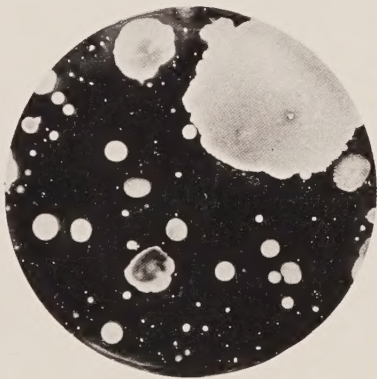
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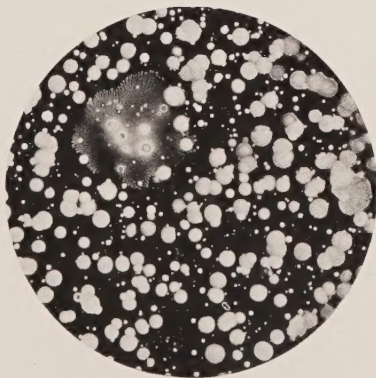
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6.

EXPLANATION OF PLATES.

Plate I.

- Fig. 1. *Staphylococcus pyogenes aureus*, a pus-forming coccus; magnified 1000 times.
Fig. 2. *Azotobacter chroococcum*, a non-symbiotic nitrogen fixing coccus; magnified 1000 times.
Fig. 3. *Bacterium mycoides*, a denitrifying bacterium (rod type) found in soil; magnified 1000 times.
Fig. 4. *Spirillum rubrum*, a harmless spirillum found in water; magnified 1000 times.
Fig. 5. *Saccharomyces cerevisiae*, the yeast cells from compressed yeast; magnified 1000 times.
Fig. 6. Spores of *Penicillium glaucum*, the common blue mold on canned fruit; magnified 1000 times.

Plate II.

- Fig. 1. Colony of *Bacterium mycoides*.
Fig. 2. Colony of *Bacterium tuberculosis* (human).
Fig. 3. Colony of *Pseudomonas medicaginis*, the cause of the stem blight of alfalfa.
Fig. 4. Colony of *Bacterium diphtheriae* (human).
Fig. 5. Colony of *Sphaerella rubina*, a fungus or mold causing the spur blight of red raspberries.
Fig. 6. Colony of *Azotobacter chroococcum*.

Plate III.

- Fig. 1. A *pure culture* of *Bacillus amylovorus*, the germ of pear blight. Note the similarity of the colonies in a pure culture.
Fig. 2. A *mixed culture*. Note the unlike character of the colonies in a mixed culture.
Fig. 3. Colonies of bacteria from soil.
Fig. 4. Colonies of bacteria from water.
Fig. 5. Colonies of bacteria and molds from dust.
Fig. 6. Colonies of bacteria from air.

